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PROVISIONAL APPLICATION FOR PATENT COVER SHEET (Large Entity)

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

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<input type="checkbox"/> Additional inventors are being named on page 2 attached hereto					
TITLE OF THE INVENTION (280 characters max)					
GLP-1 ANALOGUES					
CORRESPONDENCE ADDRESS					
Direct all correspondence to:					
<input type="checkbox"/> Customer Number <input type="text"/> → <div>Place Customer Number Bar Code Label here</div>					
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ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/>	Specification	Number of Pages	24		
<input type="checkbox"/>	Drawing(s)	Number of Sheets	<input checked="" type="checkbox"/> Other (specify) <input type="text"/>		
Claims: 7 pgs; Abstract: 1 pg.					
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input checked="" type="checkbox"/> No.					
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: <input type="text"/>					

Respectfully submitted,

SIGNATURE



DATE

December 7, 1998

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, DC 20231

Patent
Attorney Docket No.: BPC077P

**PROVISIONAL APPLICATION
UNDER 37 CFR 1.53(b)(2)**

TITLE: GLP-1 ANALOGUES

APPLICANTS: ZHENG XIN DONG and DAVID H. COY

EXPRESS MAIL LABEL NO. EE788788301US

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GLP-1 ANALOGUES

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Background of the Invention

The present invention is directed to peptide analogues of glucagon-like peptide-1, the pharmaceutically-acceptable salts thereof, to methods of using such analogues to treat mammals and to pharmaceutical compositions useful therefor comprising said analogues.

10 Glucagon-like peptide-1 (7-36) amide (GLP-1) is synthesized in the intestinal L-cells by tissue-specific post-translational processing of the glucagon precursor pre-proglucagon (Varndell, J.M., et al., J. Histochem Cytochem, 1985:33:1080-6) and is released into the circulation in response to a meal. The plasma concentration of GLP-1 rises from a fasting level of approximately 15 pmol/L to a peak postprandial level of 40 pmol/L. It has been demonstrated that, for a given rise in plasma glucose concentration, the increase in plasma
15 insulin is approximately threefold greater when glucose is administered orally compared with intravenously (Kreymann, B., et al., Lancet 1987:2, 1300-4). This alimentary enhancement of insulin release, known as the incretin effect, is primarily humoral and GLP-1 is now thought to be the most potent physiological incretin in humans. In addition to the insulinotropic effect, GLP-1 suppresses glucagon secretion, delays gastric emptying
20 (Wettergren A., et al., Dig Dis Sci 1993:38:665-73) and may enhance peripheral glucose disposal (D'Alessio, D.A. et al., J. Clin Invest 1994:93:2293-6).

In 1994, the therapeutic potential of GLP-1 was suggested following the observation that a single subcutaneous (s/c) dose of GLP-1 could completely normalize postprandial glucose levels in patients with non-insulin-dependent diabetes mellitus (NIDDM) (Gutniak,
25 M.K., et al., Diabetes Care 1994:17:1039-44). This effect was thought to be mediated both by increased insulin release and by a reduction in glucagon secretion. Furthermore, an intravenous infusion of GLP-1 has been shown to delay postprandial gastric emptying in patients with NIDDM (Williams, B., et al., J. Clin Endo Metab 1996:81:327-32). Unlike sulfonylureas, the insulinotropic action of GLP-1 is dependent on plasma glucose
30 concentration (Holz, G.G. 4th, et al., Nature 1993:361:362-5). Thus, the loss of GLP-1-mediated insulin release at low plasma glucose concentration protects against severe hypoglycemia. This combination of actions gives GLP-1 unique potential therapeutic advantages over other agents currently used to treat NIDDM.

Numerous studies have shown that when given to healthy subjects, GLP-1 potently
35 influences glycemic levels as well as insulin and glucagon concentrations (Orskov, C,

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Diabetologia 35:701-711, 1992; Holst, J.J., et al., Potential of GLP-1 in diabetes management in Glucagon III, Handbook of Experimental Pharmacology, Lefevbre PJ, Ed. Berlin, Springer Verlag, 1996, p. 311-326), effects which are glucose dependent (Kreymann, B., et al., Lancet ii: 1300-1304, 1987; Weir, G.C., et al., Diabetes 38:338-342, 1989). Moreover, it is also effective in patients with diabetes (Gutniak, M., N. Engl J Med 226:1316-1322, 1992; Nathan, D.M., et al., Diabetes Care 15:270-276, 1992), normalizing blood glucose levels in type 2 diabetic subjects (Nauck, M.A., et al., Diabetologia 36:741-744, 1993), and improving glycemic control in type 1 patients (Creutzfeldt, W.O., et al., Diabetes Care 19:580-586, 1996), raising the possibility of its use as a therapeutic agent.

GLP-1 is, however, metabolically unstable, having a plasma half-life ($t_{1/2}$) of only 1-2 min *in vivo*. Exogenously administered GLP-1 is also rapidly degraded (Deacon, C.F., et al., Diabetes 44:1126-1131, 1995). This metabolic instability limits the therapeutic potential of native GLP-1. Hence, there is a need for GLP-1 analogues that are more active or are more metabolically stable than native GLP-1.

Summary of the Invention

In one aspect, the present invention is directed to a compound of formula (I),
(R²R³)-A⁷-A⁸-A⁹-A¹⁰-A¹¹-A¹²-A¹³-A¹⁴-A¹⁵-A¹⁶-A¹⁷-A¹⁸-A¹⁹-A²⁰-A²¹-A²²-A²³-A²⁴-A²⁵-A²⁶-A²⁷-A²⁸-A²⁹-A³⁰-A³¹-A³²-A³³-A³⁴-A³⁵-A³⁶-A³⁷-R¹,

(I)

wherein

A⁷ is L-His, Ura, Paa, Pta, D-His, Tyr, 3-Pal, 4-Pal, Hppa, Tma-His, Amp or deleted, provided that when A⁷ is Ura, Paa, Pta or Hppa then R² and R³ are deleted;

A⁸ is Ala, D-Ala, Aib, Acc, N-Me-Ala, N-Me-D-Ala, Arg or N-Me-Gly;

A⁹ is Glu, N-Me-Glu, N-Me-Asp or Asp;

A¹⁰ is Gly, Acc, Ala, D-Ala, Phe or Aib;

A¹¹ is Thr or Ser;

A¹² is Phe, Acc, Aic, Aib, 3-Pal, 4-Pal, β -Nal, Cha, Trp or X¹-Phe;

A¹³ is Thr or Ser;

A¹⁴ is Ser, Thr, Ala or Aib;

A¹⁵ is Asp, Ala, D-Asp or Glu;

A¹⁶ is Val, D-Val, Acc, Aib, Leu, Ile, Tie, Nle, Abu, Ala, D-Ala, Tba or Cha;

A¹⁷ is Ser, Ala, D-Ala, Aib, Acc or Thr;

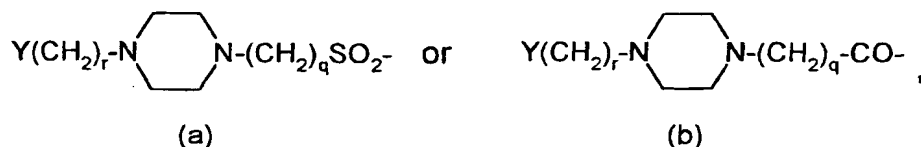
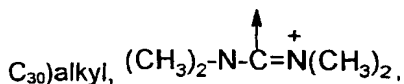
A¹⁸ is Ser, Ala, D-Ala, Aib, Acc or Thr;

A¹⁹ is Tyr, D-Tyr, Cha, Phe, 3-Pal, 4-Pal, Acc, β -Nal, Amp or X¹-Phe;

- A²⁰ is Leu, Ala, Acc, Aib, Nle, Ile, Cha, Tle, Val, Phe or X¹-Phe;
A²¹ is Glu, Ala or Asp;
A²² is Gly, Acc, Ala, D-Ala, β-Ala or Aib;
A²³ is Gln, Asp, Ala, D-Ala, Aib, Acc, Asn or Glu;
5 A²⁴ is Ala, Aib, Val, Abu, Tle or Acc;
A²⁵ is Ala, Aib, Val, Abu, Tle, Acc, Lys, Arg, hArg, Orn, HN-CH((CH₂)_n-NR¹⁰R¹¹)-C(O) or HN-CH((CH₂)_e-X³)-C(O);
A²⁶ is Lys, Ala, 3-Pal, 4-Pal, Arg, hArg, Orn, Amp, HN-CH((CH₂)_n-NR¹⁰R¹¹)-C(O) or HN-CH((CH₂)_e-X³)-C(O);
10 A²⁷ is Glu, Ala, D-Ala or Asp;
A²⁸ is Phe, Ala, Pal, β-Nal, X¹-Phe, Aic, Acc, Aib, Cha or Trp;
A²⁹ is Ile, Acc, Aib, Leu, Nle, Cha, Tle, Val, Abu, Ala, Tba or Phe;
A³⁰ is Ala, Aib, Acc or deleted;
A³¹ is Trp, Ala, β-Nal, 3-Pal, 4-Pal, Phe, Acc, Aib, Cha, Amp or deleted;
15 A³² is Leu, Ala, Acc, Aib, Nle, Ile, Cha, Tle, Phe, X¹-Phe, Ala or deleted;
A³³ is Val, Acc, Aib, Leu, Ile, Tle, Nle, Cha, Ala, Phe, Abu, X¹-Phe, Tba, Gaba or deleted;
A³⁴ is Lys, Arg, hArg, Orn, Amp, Gaba, HN-CH((CH₂)_n-NR¹⁰R¹¹)-C(O), HN-CH((CH₂)_e-X³)-C(O) or deleted;
A³⁵ is Gly or deleted;
20 A³⁶ is L- or D-Arg, D- or L-Lys, D- or L-hArg, D- or L-Orn, Amp, HN-CH((CH₂)_n-NR¹⁰R¹¹)-C(O), HN-CH((CH₂)_e-X³)-C(O) or deleted;
A³⁷ is Gly or deleted;
X¹ for each occurrence is independently selected from the group consisting of (C₁-C₆)alkyl, OH and halo;
25 R¹ is OH, NH₂, (C₁-C₁₂)alkoxy, or NH-X²-CH₂-Z, wherein X² is a (C₁-C₁₂)hydrocarbon moiety, and Z is H, OH, CO₂H or CONH₂;
- $$\text{X}^4 - \text{N} \begin{array}{c} \diagup \quad \diagdown \\ \diagdown \quad \diagup \end{array} \text{N} - (\text{CH}_2)_f - \text{CH}_3$$
- X³ is or -C(O)-NHR¹², wherein X⁴ for each occurrence is independently -C(O)-, -NH-C(O)- or -CH₂-, and f for each occurrence is independently an integer from 1 to 29;
30 each of R² and R³ is independently selected from the group consisting of H, (C₁-C₃₀)alkyl, (C₂-C₃₀)alkenyl, phenyl(C₁-C₃₀)alkyl, naphthyl(C₁-C₃₀)alkyl, hydroxy(C₁-C₃₀)alkyl, hydroxy(C₂-C₃₀)alkenyl, hydroxyphenyl(C₁-C₃₀)alkyl, and

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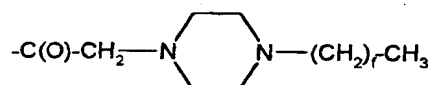
hydroxynaphthyl(C₁-C₃₀)alkyl; or one of R² and R³ is C(O)X⁵ in which X⁵ is (C₁-C₃₀)alkyl, (C₂-C₃₀)alkenyl, phenyl(C₁-C₃₀)alkyl, naphthyl(C₁-C₃₀)alkyl, hydroxy(C₁-C₃₀)alkyl, hydroxy(C₂-C₃₀)alkenyl, hydroxyphenyl(C₁-C₃₀)alkyl, hydroxynaphthyl(C₁-



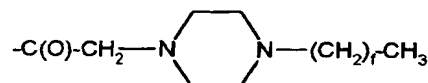
where Y is H or OH, r is 0-4 and q is 0-4;

n for each occurrence is independently an integer from 1-5; and

R¹⁰ and R¹¹ for each occurrence is each independently H, (C₁-C₃₀)alkyl, (C₁-C₃₀)acyl, (C₁-C₃₀)alkylsulfonyl, -C((NH)(NH₂)) or



, provided that when R¹⁰ is (C₁-C₃₀)acyl, (C₁-



C₃₀)alkylsulfonyl, -C((NH)(NH₂)) or (C₁-C₃₀)alkyl; and

R¹² is (C₁-C₃₀)alkyl;

, R¹¹ is H or

with the proviso that:

- 15 (i) at least one amino acid of a compound of formula (I) is not the same as the native sequence of hGLP-1(7-36, or -37)NH₂ or hGLP-1(7-36, or -37)OH;
- (ii) a compound of formula (I) is not an analogue of hGLP-1(7-36, or -37)NH₂ or hGLP-1(7-36, or -37)OH wherein a single position has been substituted by Ala;
- (iii) a compound of formula (I) is not [Lys²⁶(N^ε-alkanoyl)]hGLP-1(7-36, or -37)-E, [Lys³⁴(N^ε-alkanoyl)]hGLP-1(7-36, or -37)-E, [Lys^{26,34}-bis(N^ε-alkanoyl)]hGLP-1(7-36, or -37)-E, [Arg²⁶, Lys³⁴(N^ε-alkanoyl)]hGLP-1(8-36, or -37)-E, or [Arg^{26,34}, Lys³⁶(N^ε-alkanoyl)]hGLP-1(7-36, or -37)-E, wherein E is -OH or -NH₂;
- (iv) a compound of formula (I) is not Z-hGLP-1(7-36, or -37)-OH, Z-hGLP-1(7-36, or -37)-NH₂, where Z is selected from the group consisting of
- 25 (a) [Arg²⁶], [Arg³⁴], [Arg^{26,34}], [Lys³⁶], [Arg²⁶, Lys³⁶], [Arg³⁴, Lys³⁶], [D-Lys³⁶], [Arg³⁶], [D-Arg³⁶], [Arg^{26,34}, Lys³⁶] or [Arg^{26,36}, Lys³⁴];

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- (b) [Asp²¹];
 (c) at least one of [Aib⁸], [D-Ala⁸] and [Asp⁹]; and
 (d) [Tyr⁷], [N-acyl-His⁷], [N-alkyl-His⁷], [N-acyl-D-His⁷] or [N-alkyl-D-His⁷];
 (v) a compound of formula (I) is not a combination of any two of the substitutions listed in
 5 groups (a) to (d); and
 (vi) a compound of formula (I) is not [N-Me-Ala⁸]hGLP-1(8-36 or -37), [Glu¹⁵]hGLP-1(7-36 or
 -37), [Asp²¹]hGLP-1(7-36 or -37) or [Phe³¹]hGLP-1(7-36 or -37).

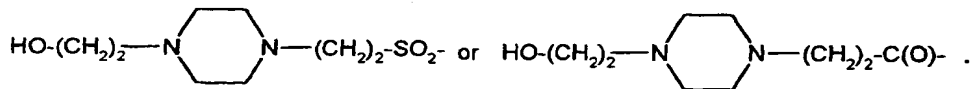
A preferred compound of the immediately foregoing compound of formula (I) is
 where A¹¹ is Thr; A¹³ is Thr; A¹⁴ is Ser, Aib or Ala; A¹⁷ is Ser, Ala, Aib or D-Ala; A¹⁸ is Ser,
 10 Ala, Aib or D-Ala; A²¹ is Glu or Ala; A²³ is Gln, Glu, or Ala; and A²⁷ is Glu or Ala; or a
 pharmaceutically acceptable salt thereof.

A preferred compound of the immediately foregoing compound of formula (I) is
 where A⁹ is Glu, N-Me-Glu or N-Me-Asp; A¹² is Phe, Acc or Aic; A¹⁶ is Val, D-Val, Acc, Aib,
 Ala, Tle or D-Ala; A¹⁹ is Tyr, 3-Pal, 4-Pal or D-Tyr; A²⁰ is Leu, Acc, Cha, Ala or Tle; A²⁴ is
 15 Ala, Aib or Acc; A²⁵ is Ala, Aib, Acc, Lys, Arg, hArg, Orn, HN-CH((CH₂)_n-NH-R¹⁰)-C(O); A²⁸
 is Phe or Ala; A²⁹ is Ile, Acc or Tle; A³⁰ is Ala, Aib or deleted; A³¹ is Trp, Ala, 3-Pal, 4-Pal or
 deleted; A³² is Leu, Acc, Cha, Ala or deleted; A³³ is Val, Acc, Ala, Gaba, Tle or deleted; or a
 pharmaceutically acceptable salt thereof.

A preferred compound of the immediately foregoing compound of formula (I) is
 20 where A⁸ is Ala, D-Ala, Aib, A6c, A5c, N-Me-Ala, N-Me-D-Ala or N-Me-Gly; A¹⁰ is Gly, Ala,
 D-Ala or Phe; A¹² is Phe, A6c or A5c; A¹⁶ is Val, Ala, Tle, A6c, A5c or D-Val; A²⁰ is Leu,
 A6c, A5c, Cha, Ala or Tle; A²² is Gly, Aib, β-Ala, L-Ala or D-Ala; A²⁴ is Ala or Aib; A²⁹ is Ile,
 A6c, A5c or Tle; A³² is Leu, A6c, A5c, Cha, Ala or deleted; A³³ is Val, A6c, A5c, Ala, Gaba,
 Tle or deleted; or a pharmaceutically acceptable salt thereof.

25 A preferred compound of the immediately foregoing compound of formula (I) is
 where R¹ is OH or NH₂ or a pharmaceutically acceptable salt thereof.

A preferred compound of the immediately foregoing compound of formula (I) or a
 pharmaceutically acceptable salt thereof is where R² is H and R³ is (C₁-C₃₀)alkyl, (C₂-
 C₃₀)alkenyl, (C₁-C₃₀)acyl,



A most preferred compound of formula (I) is where said compound is

[D-Ala⁸, Ala^{17,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]-GLP-1(7-34)-NH₂; [D-Ala^{8,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂; [Ala^{18,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂; [Ala^{16,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂; [Ala^{14,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂; [Ala^{22,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂; [Hppa⁷]hGLP-1(7-36)-NH₂; [Ala^{15,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂; [Ala^{17,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂; [Ala^{22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; [Ala^{15,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; [Ala^{17,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; [Ala^{18,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; [Ala^{21,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; [Ala^{22,23,26,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; [Ala^{22,23,27,32}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; [Ala^{22,23,26,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂; [Ala^{22,23,27,31}, 3-Pal¹⁹, Gaba³³]hGLP-1(7-33)-NH₂; [Ala^{22,23,27,28}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂; [Ala^{22,23,27,29}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂; [Ala^{23,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂; [Ala^{20,22,23,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂; [Ala^{22,23,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂; [Ala^{17,22,23,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂; [D-Ala¹⁰, Ala^{22,23,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂; [D-Ala⁸, Ala^{17,23,27}, 3-Pal^{19,31}]hGLP-1(7-34)-NH₂; [Ala^{17,23,27}, 3-Pal^{19,26,31}]hGLP-1(7-34)-NH₂; [D-Ala⁸, Ala¹⁷, 3-Pal^{19,31}]hGLP-1(7-34)-NH₂; [Ala^{17,23,27}, 3-Pal^{19,31}]hGLP-1(7-34)-NH₂; [D-Ala⁸, Ala^{17,23,27}, 3-Pal^{19,31}, Tie²⁹]hGLP-1(7-34)-NH₂; [D-Ala⁸, Ala^{17,23,27}, 3-Pal^{19,31}, Tie¹⁶]hGLP-1(7-34)-NH₂; [D-Ala⁸, Ala^{17,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; [D-Ala²², Ala^{17,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; [Aib⁸, Ala^{17,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; [D-Ala⁸, Ala^{17,22,23,27}, 3-Pal^{19,31}]hGLP-1(7-33)-NH₂; [Aib⁸, Ala^{17,22,23,27}, 3-Pal^{19,31}]hGLP-1(7-33)-NH₂; [Ala^{17,18,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; [Ala^{17,23,27}, 3-Pal^{19,31}, Tie³³, Gaba³⁴]hGLP-1(7-34)-NH₂; [Tie¹⁶, Ala^{17,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; [N-Me-D-Ala⁸, Ala^{17,22,23,27}, 3-Pal^{19,31}]hGLP-1(7-33)-NH₂; [Aib⁸, Ala^{17,18,22,23,27}, 3-Pal^{19,31}]hGLP-1(7-33)-NH₂; [Ala^{17,18,22,23,27}, 3-Pal^{19,31}, Tie^{16,20}, Gaba³⁴]hGLP-1(7-34)-NH₂; [D-Ala⁸, Ala^{17,18,22,23,27}, 3-Pal^{19,31}, Tie¹⁶, Gaba³⁴]hGLP-1(7-34)-NH₂; [D-Ala^{8,22}, Ala^{17,18,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; [D-Ala^{8,18}, Ala^{17,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; [D-Ala^{8,17}, Ala^{18,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; or [D-Ala⁸, Ala^{17,18,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; or a pharmaceutically acceptable salt thereof.

Another most preferred compound of formula (I) is wherein said compound is

[Aib⁸, A6c³²]hGLP-1(7-36)-NH₂; [A6c^{20,32}]hGLP-1(7-36)-NH₂; [Aib⁸]hGLP-1(7-36)-NH₂; [(Tma-His)⁷]hGLP-1(7-36)-NH₂; [A6c⁸]hGLP-1(8-36)-NH₂; [A6c⁸]hGLP-1(7-36)-NH₂; [A6c^{16,20}]hGLP-1(7-36)-NH₂; [A6c^{29,32}]hGLP-1(7-36)-NH₂; [A6c²⁰, Aib²⁴]hGLP-1(7-36)-NH₂; [Aib²⁴, A6c^{29,32}]hGLP-1(7-36)-NH₂; [A6c^{16,29,32}]hGLP-1(7-36)-NH₂; [Ura⁷]hGLP-1(7-36)-NH₂; [Paa⁷]hGLP-1(7-36)-NH₂; [Pta⁷]hGLP-1(7-36)-NH₂; [N-Me-Ala⁸]hGLP-1(7-36)-NH₂; [N-Me-

D-Ala⁸]hGLP-1(7-36)-NH₂; [N-Me-D-Ala⁸]hGLP-1(8-36)-NH₂; [N-Me-Gly⁸]hGLP-1(7-36)-NH₂; [A5c⁸]hGLP-1(7-36); [N-Me-Glu⁹]hGLP-1(7-36)-NH₂; [A5c⁸, A6c^{20,32}]hGLP-1(7-36)-NH₂; [Aib⁸, A6c³²]hGLP-1(7-36)-NH₂; [Aib^{8,25}]hGLP-1(7-36)-NH₂; [Aib^{8,24}]hGLP-1(7-36)-NH₂; [Aib^{8,30}]hGLP-1(7-36)-NH₂; [Aib⁸, Cha²⁰]hGLP-1(7-36)-NH₂; [Aib⁸, Cha³²]hGLP-1(7-36)-NH₂; [Aib⁸, Glu²³]hGLP-1(7-36)-NH₂; [Aib⁸, A6c²⁰]hGLP-1(7-36)-NH₂; [Aib⁸, A6c^{20,32}]hGLP-1(7-36)-NH₂; [Aib^{8,22}]hGLP-1(7-36)-NH₂; [Aib⁸, β-Ala²²]hGLP-1(7-36)-NH₂; [Aib⁸, Lys²⁵]hGLP-1(7-36)-NH₂; [Aib⁸, A6c¹²]hGLP-1(7-36)-NH₂; [Aib⁸, A6c²⁹]hGLP-1(7-36)-NH₂; [Aib⁸, A6c³³]hGLP-1(7-36)-NH₂; [Aib^{8,14}]hGLP-1(7-36)-NH₂; [Aib^{8,18}]hGLP-1(7-36)-NH₂; or [Aib^{8,17}]hGLP-1(7-36)-NH₂; or a pharmaceutically acceptable salt thereof.

In another aspect, the present invention provides a pharmaceutical composition comprising an effective amount of a compound of formula (I) as defined hereinabove or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier or diluent.

In still another aspect, the present invention provides a method of eliciting an agonist effect from a GLP-1 receptor in a subject in need thereof which comprises administering to said subject an effective amount of a compound of formula (I) as defined hereinabove or a pharmaceutically acceptable salt thereof.

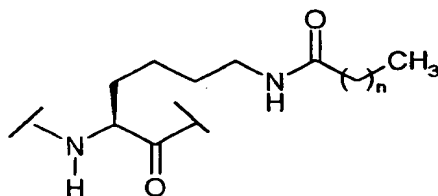
In yet a further aspect, this invention provides a method of treating a disease selected from the group consisting of Type I diabetes, Type II diabetes, obesity, glucagonomas, secretory disorders of the airway, metabolic disorder, arthritis, osteoporosis, central nervous system disease, restenosis and neurodegenerative disease, in a subject in need thereof which comprises administering to said subject an effective amount of a compound of formula (I) as defined hereinabove or a pharmaceutically acceptable salt thereof. Preferred of the foregoing method is where the disease is Type I diabetes or Type II diabetes.

With the exception of the N-terminal amino acid, all abbreviations (e.g. Ala) of amino acids in this disclosure stand for the structure of -NH-CH(R)-CO-, wherein R is the side chain of an amino acid (e.g., CH₃ for Ala). For the N-terminal amino acid, the abbreviation stands for the structure of (R²R³)-N-CH(R)-CO-, wherein R is a side chain of an amino acid and R² and R³ are as defined above except in the case where A⁷ is Ura, Paa, Pta or Hppa in which case R² and R³ are not present since Ura, Paa, Pta and Hppa are considered here as des-amino amino acids. The abbreviations: β-Nal, Nle, Cha, Amp, 3-Pal, 4-Pal and Aib stand for the following α-amino acids: β-(2-naphthyl)alanine, norleucine, cyclohexylalanine, 4-amino-phenylalanine, β-(3-pyridinyl)alanine, β-(4-pyridinyl)alanine and α-aminoisobutyric

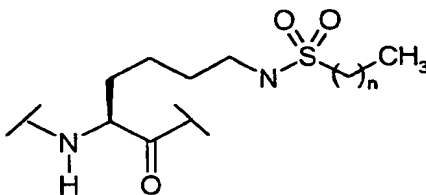
acid, respectively. Other amino acid definitions are: Ura is urocanic acid; Pta is (4-pyridylthio) acetic acid; Paa is *trans*-3-(3-pyridyl) acrylic acid; Tma-His is N,N-tetramethylamidino-histidine; N-Me-Ala is N-methyl-alanine; N-Me-Gly is N-methyl-glycine; N-Me-Glu is N-methyl-glutamic acid; Tle is *tert*-butylglycine; Abu is α -aminobutyric acid; Tba is *tert*-butylalanine; Orn is ornithine; Aib is α -aminoisobutyric acid; β -Ala is β -alanine; Gaba is γ -aminobutyric acid; Ava is 5-aminovaleric acid; and Aic is 2-aminoindane-2-carboxylic acid.

What is meant by Acc is an amino acid selected from the group of 1-amino-1-cyclopropanecarboxylic acid (A3c); 1-amino-1-cyclobutanecarboxylic acid (A4c); 1-amino-1-cyclopentanecarboxylic acid (A5c); 1-amino-1-cyclohexanecarboxylic acid (A6c); 1-amino-1-cycloheptanecarboxylic acid (A7c); 1-amino-1-cyclooctanecarboxylic acid (A8c); and 1-amino-1-cyclononanecarboxylic acid (A9c). In the above formula, hydroxyalkyl, hydroxyphenylalkyl, and hydroxynaphthylalkyl may contain 1-4 hydroxy substituents. COX⁵ stands for -C=O·X⁵. Examples of -C=O·X⁵ include, but are not limited to, acetyl and phenylpropionyl.

What is meant by Lys(N^ε-alkanoyl) is represented by the following structure:



What is meant by Lys(N^ε-alkylsulfonyl) is represented

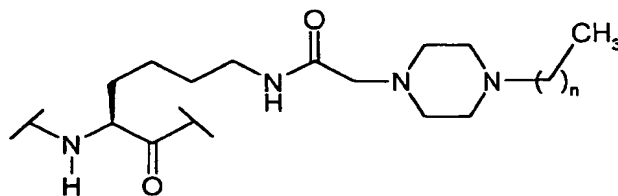


by the following structure:

(2-(4-alkyl-1-piperazine)-acetyl))

is represented

What is meant by Lys(N^ε-
by the following



20 structure:

alkyl-piperazine))

is

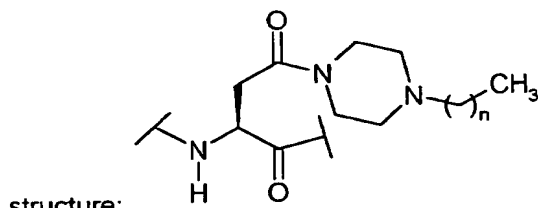
represented

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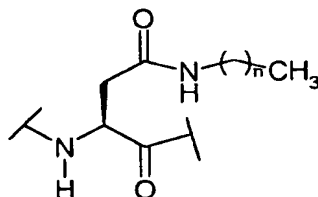
the

following

What is meant by Asp(1-(4-



What is meant by Asp(1-alkylamino) is



represented by the following structure:
foregoing structures is 1 to 30.

The variable n in the

The full names for other abbreviations used herein are as follows: Boc for t-butylloxycarbonyl, HF for hydrogen fluoride, Fm for formyl, Xan for xanthyl, Bzl for benzyl, Tos for tosyl, DNP for 2,4-dinitrophenyl, DMF for dimethylformamide, DCM for dichloromethane, HBTU for 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate, DIEA for diisopropylethylamine, HOAc for acetic acid, TFA for trifluoroacetic acid, 2CIZ for 2-chlorobenzyloxycarbonyl and OcHex for O-cyclohexyl.

A peptide of this invention is also denoted herein by another format, e.g., [A5c⁸]hGLP-1(7-36)NH₂, with the substituted amino acids from the natural sequence placed between the set of brackets (e.g., A5c⁸ for Ala⁸ in hGLP-1). The abbreviation GLP-1 means glucagon-like peptide-1. The numbers between the parentheses refer to the number of amino acids present in the peptide (e.g., hGLP-1(7-36) is amino acids 7 through 36 of the peptide sequence for human GLP-1). The sequence for hGLP-1(7-37) is listed in Mojsov, S., Int. J. Peptide Protein Res., 40, 1992, pp. 333-342. The designation "NH₂" in hGLP-1(7-36)NH₂ indicates that the C-terminus of the peptide is amidated. hGLP-1(7-36) means that the C-terminus is the free acid.

Detailed Description

The peptides of this invention can be prepared by standard solid phase peptide synthesis. See, e.g., Stewart, J.M., et al., Solid Phase Synthesis (Pierce Chemical Co., 2d ed. 1984). The substituents R² and R³ of the above generic formula can be attached to the free amine of the N-terminal amino acid by standard methods known in the art. For example, alkyl groups, e.g., (C₁-C₃₀)alkyl, can be attached using reductive alkylation. Hydroxyalkyl groups, e.g., (C₁-C₃₀)hydroxyalkyl, can also be attached using reductive alkylation wherein the free hydroxy group is protected with a t-butyl ester. Acyl groups, e.g.,

COE¹, may be attached by coupling the free acid, e.g., E¹COOH, to the free amine of the N-terminal amino acid by mixing the completed resin with 3 molar equivalents of both the free acid and diisopropylcarbodiimide in methylene chloride for one hour. If the free acid contains a free hydroxy group, e.g., p-hydroxyphenylpropionic acid, then the coupling should be performed with an additional 3 molar equivalents of HOBT.

When R¹ is NH-X²-CH₂-Z (Z=CONH₂), the synthesis of the peptide starts with BocHN-X²-CH₂-COOH which is coupled to the MBHA resin. If R¹ is NH-X²-CH₂-COOH (Z=COOH) the synthesis of the peptide starts with Boc-HN-X²-CH₂-COOH which is coupled to PAM resin.

The following describes a synthetic method for making a peptide of this invention, which method is well-known to those skilled in the art. Other methods are also known to those skilled in the art.

Benzhydrylamine-polystyrene resin (Advanced ChemTech, Inc., Louisville, KY) (0.9 g, 0.3 mmole) in the chloride ion form is placed in a reaction vessel of an Advanced ChemTech Peptide Synthesizer Model 200 programmed to perform the following reaction cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid in methylene chloride (2 times for 1 and 15 min each); (c) methylene chloride; (d) ethanol; (e) methylene chloride; (f) 10% diisopropylethylamine in methylene chloride.

The neutralized resin is stirred with Boc-protected amino acid which is to be the C-terminal amino acid of the desired peptide to be synthesized and diisopropylcarbodiimide (3 mmole each) in methylene chloride for 1 hour and the resulting amino acid resin is then cycled through steps (a) through (f) in the above wash program. The other amino acids (3 mmol) of the desired peptide are then coupled successively by the same procedure. The finished peptide is cleaved from the resin by mixing it with anisole (5 ml), dithiothreitol (100 mg) and anhydrous hydrogen fluoride (35 ml) at about 0 °C and stirring it for about 45 min. Excess hydrogen fluoride is evaporated rapidly under a stream of dry nitrogen and free peptide precipitated and washed with ether. The crude peptide is then dissolved in a minimum volume of dilute acetic acid and eluted on a column (2.5 x 25 cm) of VYDAC® octadecylsilane silica (10 mM) and eluted with a linear gradient of 20-60% acetonitrile over about 1 h in 0.1% trifluoroacetic acid in water. Fractions are examined by thin layer chromatography and analytical high performance liquid chromatography (40-70% B at 1%/min, solution B is 80% acetonitrile/water containing 0.1% TFA) and pooled to give maximum purity rather than yield. Repeated lyophilization of the solution from water gives the product as a white, fluffy powder.

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The product peptide is analyzed by HPLC. Amino acid analysis of an acid hydrolysate of the product peptide can confirm the composition of the peptide. Laser desorption MS is used to determine the molecular weight of the peptide.

5 The protected amino acid 1-[N-tert-butoxycarbonyl-amino]-1-cyclohexane-carboxylic acid (Boc-A6c-OH) was synthesized as follows. 19.1 g (0.133 mol) of 1-amino-1-cyclohexanecarboxylic acid (Acros Organics, Fisher Scientific, Pittsburgh, PA) was dissolved in 200 ml of dioxane and 100 ml of water. To it was added 67 ml of 2N NaOH. The solution was cooled in an ice-water bath. 32.0 g (0.147 mol) of di-tert-butyl-dicarbonate was added to this solution. The reaction mixture was stirred overnight at room
10 temperature. Dioxane was then removed under reduced pressure. 200 ml of ethyl acetate was added to the remaining aqueous solution. The mixture was cooled in an ice-water bath. The pH of the aqueous layer was adjusted to about 3 by adding 4N HCl. The organic layer was separated. The aqueous layer was extracted with ethyl acetate (1 x 100 ml). The two organic layers were combined and washed with water (2 x 150 ml), dried over
15 anhydrous MgSO_4 , filtered, and concentrated to dryness under reduced pressure. The residue was recrystallized in ethyl acetate/hexanes. 9.2 g of the pure product was obtained. 29% yield.

Boc-A5c-OH was synthesized in an analogous manner to that of Boc-A6c-OH. Other protected Acc amino acids can be prepared in an analogous manner by a person of
20 ordinary skill in the art as enabled by the teachings herein.

In the synthesis of a peptide of this invention containing A5c, A6c and/or Aib, the coupling time is about 2 hrs. for these residues and the residue immediately following them. For the synthesis of [Tma-His⁷]hGLP-1(7-36) NH_2 , HBTU (2 mmol) and DIEA (1.0 ml) in 4 ml DMF were used to react with the N-terminal free amine of the peptide-resin in the last
25 coupling reaction; the coupling time is about 2 hours.

The full names for the abbreviations used above are as follows: Boc for t-butyloxycarbonyl, HF for hydrogen fluoride, Fm for formyl, Xan for xanthyl, Bzl for benzyl, Tos for tosyl, DNP for 2,4-dinitrophenyl, DMF for dimethylformamide, DCM for dichloromethane, HBTU for 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyl uronium
30 hexafluorophosphate, DIEA for diisopropylethylamine, HOAc for acetic acid, TFA for trifluoroacetic acid, 2CIZ for 2-chlorobenzoyloxycarbonyl, 2BrZ for 2-bromobenzoyloxycarbonyl and OcHex for O-cyclohexyl.

The substituents R^2 and R^3 of the above generic formula can be attached to the free amine of the N-terminal amino acid by standard methods known in the art. For example,

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alkyl groups, e.g., (C₁-C₃₀)alkyl, may be attached using reductive alkylation. Hydroxyalkyl groups, e.g., (C₁-C₃₀)hydroxyalkyl, can also be attached using reductive alkylation wherein the free hydroxy group is protected with a t-butyl ester. Acyl groups, e.g., COX¹, can be attached by coupling the free acid, e.g., X¹COOH, to the free amine of the N-terminal amino acid by mixing the completed resin with 3 molar equivalents of both the free acid and diisopropylcarbodiimide in methylene chloride for about one hour. If the free acid contains a free hydroxy group, e.g., p-hydroxyphenylpropionic acid, then the coupling should be performed with an additional 3 molar equivalents of HOBT.

A compound of the present invention can be tested for activity as a GLP-1 binding compound according to the following procedure.

Cell Culture:

RIN 5F rat insulinoma cells (ATCC-# CRL-2058, American Type Culture Collection, Manassas, VA), expressing the GLP-1 receptor, were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, and maintained at about 37 °C in a humidified atmosphere of 5% CO₂/95% air.

Radioligand Binding:

Membranes were prepared for radioligand binding studies by homogenization of the RIN cells in 20 ml of ice-cold 50 mM Tris-HCl with a Brinkman Polytron (Westbury, NY) (setting 6, 15 sec). The homogenates were washed twice by centrifugation (39,000 g / 10 min), and the final pellets were re-suspended in 50 mM Tris-HCl, containing 2.5 mM MgCl₂, 0.1 mg/ml bacitracin (Sigma Chemical, St. Louis, MO), and 0.1% BSA. For assay, aliquots (0.4 ml) were incubated with 0.05 nM [¹²⁵I]GLP-1(7-36) (~2200 Ci/mmol, New England Nuclear, Boston, MA), with and without 0.05 ml of unlabeled competing test peptides. After a 100 min incubation (25 °C), the bound [¹²⁵I]GLP-1(7-36) was separated from the free by rapid filtration through GF/C filters (Brandel, Gaithersburg, MD), which had been previously soaked in 0.5% polyethyleneimine. The filters were then washed three times with 5 ml aliquots of ice-cold 50 mM Tris-HCl, and the bound radioactivity trapped on the filters was counted by gamma spectrometry (Wallac LKB, Gaithersburg, MD). Specific binding was defined as the total [¹²⁵I]GLP-1(7-36) bound minus that bound in the presence of 1000 nM GLP1(7-36) (Bachem, Torrence, CA).

The peptides of this invention can be provided in the form of pharmaceutically acceptable salts. Examples of such salts include, but are not limited to, those formed with organic acids (e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, methanesulfonic, toluenesulfonic, or pamoic acid), inorganic acids (e.g., hydrochloric acid,

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5 sulfuric acid, or phosphoric acid), and polymeric acids (e.g., tannic acid, carboxymethyl cellulose, polylactic, polyglycolic, or copolymers of polylactic-glycolic acids). A typical method of making a salt of a peptide of the present invention is well known in the art and can be accomplished by standard methods of salt exchange. Accordingly, the TFA salt of a peptide of the present invention (the TFA salt results from the purification of the peptide by using preparative HPLC, eluting with TFA containing buffer solutions) can be converted into another salt, such as an acetate salt by dissolving the peptide in a small amount of 0.25 N acetic acid aqueous solution. The resulting solution is applied to a semi-prep HPLC column (Zorbax, 300 SB, C-8). The column is eluted with (1) 0.1N ammonium acetate aqueous solution for 0.5 hrs., (2) 0.25N acetic acid aqueous solution 0.5 hrs. and (3) a linear gradient (20% to 100% of solution B over 30 min.) at a flow rate of 4 ml/min (solution A is 0.25N acetic acid aqueous solution; solution B is 0.25N acetic acid in acetonitrile/water, 80:20). The fractions containing the peptide are collected and lyophilized to dryness.

15 As is well known to those skilled in the art, the known and potential uses of GLP-1 is varied and multitudinous [See, Todd, J.F., et al., Clinical Science, 1998, 95, pp. 325-329; and Todd, J.F. et al., European Journal of Clinical Investigation, 1997, 27, pp.533-536]. Thus, the administration of the compounds of this invention for purposes of eliciting an agonist effect can have the same effects and uses as GLP-1 itself. These varied uses of GLP-1 may be summarized as follows, treatment of: Type I diabetes, Type II diabetes, obesity, glucagonomas, secretory disorders of the airway, metabolic disorder, arthritis, osteoporosis, central nervous system diseases, restenosis and neurodegenerative diseases. GLP-1 analogues of the present invention that elicit an antagonist effect from a subject can be used for treating the following: hypoglycemia and malabsorption syndrome associated with gastroectomy or small bowel resection.

25 Accordingly, the present invention includes within its scope pharmaceutical compositions comprising, as an active ingredient, at least one of the compounds of formula (I) in association with a pharmaceutically acceptable carrier or diluent.

30 The dosage of active ingredient in the compositions of this invention may be varied; however, it is necessary that the amount of the active ingredient be such that a suitable dosage form is obtained. The selected dosage depends upon the desired therapeutic effect, on the route of administration, and on the duration of the treatment. In general, an effective dosage for the activities of this invention is in the range of 1×10^{-7} to 200 mg/kg/day, preferably 1×10^{-4} to 100 mg/kg/day, which can be administered as a single dose or divided into multiple doses.

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The compounds of this invention can be administered by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous or subcutaneous injection, or implant), nasal, vaginal, rectal, sublingual or topical routes of administration and can be formulated with pharmaceutically acceptable carriers to provide dosage forms appropriate for each route of administration.

Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, the active compound is admixed with at least one inert pharmaceutically acceptable carrier such as sucrose, lactose, or starch. Such dosage forms can also comprise, as is normal practice, additional substances other than such inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, the elixirs containing inert diluents commonly used in the art, such as water. Besides such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring and perfuming agents.

Preparations according to this invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilized by, for example, filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions. They can also be manufactured in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use.

Compositions for rectal or vaginal administration are preferably suppositories which may contain, in addition to the active substance, excipients such as coca butter or a suppository wax.

Compositions for nasal or sublingual administration are also prepared with standard excipients well known in the art.

Further, a compound of this invention can be administered in a sustained release composition such as those described in the following patents and patent applications. U.S.

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Patent No. 5,672,659 teaches sustained release compositions comprising a bioactive agent and a polyester. U.S. Patent No. 5,595,760 teaches sustained release compositions comprising a bioactive agent in a gelable form. U.S. Application No. 08/929,363 filed September 9, 1997, teaches polymeric sustained release compositions comprising a bioactive agent and chitosan. U.S. Application No. 08/740,778 filed November 1, 1996, teaches sustained release compositions comprising a bioactive agent and cyclodextrin. U.S. Application No. 09/015,394 filed January 29, 1998, teaches absorbable sustained release compositions of a bioactive agent. U.S. Application No. 09/121,653 filed July 23, 1998, teaches a process for making microparticles comprising a therapeutic agent such as a peptide in an oil-in-water process. U.S. Application No. 09/131,472 filed August 10, 1998, teaches complexes comprising a therapeutic agent such as a peptide and a phosphorylated polymer. U.S. Application No. 09/184,413 filed November 2, 1998, teaches complexes comprising a therapeutic agent such as a peptide and a polymer bearing a non-polymerizable lactone. The teachings of the foregoing patents and applications are incorporated herein by reference.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Also, all publications, patent applications, patents and other references mentioned herein are incorporated by reference.

The following examples describe synthetic methods for making a peptide of this invention, which methods are well-known to those skilled in the art. Other methods are also known to those skilled in the art. The examples are provided for the purpose of illustration and is not meant to limit the scope of the present invention in any manner.

Example 1

[D-Ala⁸, Ala^{17,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]-GLP-1(7-34)NH₂

Benzhydrylamine-polystyrene resin (Advanced ChemTech, Inc. Louisville, KY) (0.9 g, 0.3 mmole) in the chloride ion form was placed in a reaction vessel of an Advanced ChemTech peptide synthesizer Model 200 programmed to perform the following reaction cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid in methylene chloride (2 times for 1 and 15 min each); (c) methylene chloride; (d) ethanol; (e) methylene chloride; (f) 10% diisopropylethylamine in methylene chloride.

The neutralized resin was stirred with Boc-Gaba and diisopropylcarbodiimide (3 mmole each) in methylene chloride for 1 hour and the resulting amino acid resin was then cycled through steps (a) to (f) in the above wash program. The following amino acids (3

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mmole) were then coupled successively by the same procedure: Boc-Val, Boc-Leu, Boc-3-Pal, Boc-Ala, Boc-Ile, Boc-Phe, Boc-Ala, Boc-Lys(2-Cl-Z), Boc-Ala, Boc-Ala, Boc-Ala, Boc-Ala, Boc-Glu(Bzl), Boc-Leu, Boc-3-Pal, Boc-Ser(Bzl), Boc-Ala, Boc-Val, Boc-Asp(Bzl), Boc-Ser(Bzl), Boc-Thr(Bzl), Boc-Phe, Boc-Thr(Bzl), Boc-Gly, Boc-Glu(Bzl), Boc-D-Ala, Boc-His(Bom).

The resin with the completed peptide sequence was mixed with anisole (5 ml), dithiothreitol (100 mg) and anhydrous hydrogen fluoride (35 ml) at about 0 °C and stirred for about 45 min. Excess hydrogen fluoride was evaporated rapidly under a stream of dry nitrogen and free peptide precipitated and washed with ether. The crude peptide was then dissolved in a minimum volume of dilute acetic acid and eluted on a column (2.5 x 25 cm) of VYDAC® octadecylsilane silica (10 mM) and eluted with a linear gradient of 20-60% acetonitrile over about 1 h in 0.1% trifluoroacetic acid in water. Fractions were examined by thin layer chromatography and analytical high performance liquid chromatography (40-70% B at 1%/min; r.t.: 14.1 min) and pooled to give maximum purity rather than yield. Repeated lyophilization of the solution from water gives the product (49.9 mg) as a white, fluffy powder.

The product was found to be homogeneous by HPLC and tlc. Amino acid analysis of an acid hydrolysate confirms the composition of the peptide. Laser desorption MS gave a MW of 2880 (Calc. M+H 2873).

Example 2

Synthesis of Peptide Lower-Alkylamides

Peptides are assembled on O-benzyl-polystyrene resin (often referred to as Merrifield resin) using the Boc amino acid protocol described in Example 1, except that Asp and Glu amino acid carboxyl side-chains are protected with an Fm (fluorenylmethyl ester) group. Completed peptide-resins are suspended in dilute DMF solutions of an appropriate lower alkylamine (such as ethylamine, propylamine, phenethylamine, 1,2-diaminoethane, etc.) and stirred at about 60 °C (for about 18 hrs) whereupon filtration, removal of solvents under reduced pressure and trituration of cleaved peptide oil with ether gives a solid, protected alkylamide peptide. This is then subjected to HF cleavage to remove additional side chain protecting groups and HPLC purification as described in Example 1.

Examples 3-5

Examples 3-5 can be synthesized substantially according to the procedure described in Example 1 using the appropriate protected amino acids to yield the noted peptides.

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Example 3: [Aib⁸, D-Ala¹⁷, Ala^{18,22,23,27}, 3-Pal^{19,31}, Tle¹⁶, Gaba³⁴]-GLP-1(7-34)NH₂

Example 4: [Aib⁸, D-Ala¹⁷, Ala^{22,23,27}, 3-Pal^{19,31}, Tle¹⁶]-GLP-1(7-33)NH₂

Example 5: [Aib⁸, D-Ala¹⁷, Ala^{22,23,27}, 3-Pal^{19,31}, Tle^{16,20}]-GLP-1(7-33)NH₂

Examples 6-51

5 Examples 6-51 were made substantially according to the procedure described for Example 1 but using the appropriate protected amino acid to yield the noted peptide. MS were obtained by laser desorption MS (NA means not available).

Example 6: [D-Ala^{8,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂; MS = 2971.0; Calc. MS = 2974.4.

Example 7: [Ala^{18,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂; MS = 2954.4; Calc. MS = 2958.4.

10 Example 8: [Ala^{16,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂; MS = 2943.0; Calc. MS = 2946.3.

Example 9: [Ala^{14,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂; MS = 2956.0; Calc. MS = 2958.4.

Example 10: [Ala^{22,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂; MS = 2981.0; Calc. MS = 2988.4.

Example 11: [Hppa⁷]hGLP-1(7-36)-NH₂; MS = NA

Example 12: [Ala^{15,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂; MS = 2928.0; Calc. MS = 2930.4.

15 Example 13: [Ala^{17,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂; MS = 2955.0; Calc. MS = 2958.4.

Example 14: [Ala^{22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = 2896.0; Calc. MS = 2888.3.

Example 15: [Ala^{15,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = 2852.0; Calc. MS = 2844.3.

20 Example 16: [Ala^{17,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = 2880.0; Calc. MS = 2872.3.

Example 17: [Ala^{18,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = 2870.0; Calc. MS = 2872.3.

Example 18: [Ala^{21,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = NA.

25 Example 19: [Ala^{22,23,26,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = 2832.0; Calc. MS = 2831.2.

Example 20: [Ala^{22,23,27,32}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = 2855.0; Calc. MS = 2846.2.

Example 21: [Ala^{22,23,26,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂; MS = 2729.0; Calc. MS = 2732.0.

30 Example 22: [Ala^{22,23,27,31}, 3-Pal¹⁹, Gaba³³]hGLP-1(7-33)-NH₂; MS = 2711.6; Calc. MS = 2712.0.

Example 23: [Ala^{22,23,27,28}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂; MS = 2712.0; Calc. MS = 2713.0.

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- Example 24: [Ala^{22,23,27,29}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂; MS = 2746.9; Calc. MS = 2747.1.
- Example 25: [Ala^{23,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂; MS = 2777.0; Calc. MS = 2,775.1.
- 5 Example 26: [Ala^{20,22,23,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂; MS = 2742.0; Calc. MS = 2747.1.
- Example 27: [Ala^{22,23,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂; MS = 2786.7; Calc. MS = 2789.1.
- Example 28: [Ala^{17,22,23,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂; MS = 2771.0; Calc. MS = 2773.1.
- 10 Example 29: [D-Ala¹⁰, Ala^{22,23,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂; MS = 2802.0; Calc. MS = 2803.2.
- Example 30: [D-Ala⁸, Ala^{17,23,27}, 3-Pal^{19,31}]hGLP-1(7-34)-NH₂; MS = 2905.0; Calc. MS = 2901.3.
- 15 Example 31: [Ala^{17,23,27}, 3-Pal^{19,26,31}]hGLP-1(7-34)-NH₂; MS = 2920.0; Calc. MS = 2921.3.
- Example 32: [D-Ala⁸, Ala¹⁷, 3-Pal^{19,31}]hGLP-1(7-34)-NH₂; MS = 2908.0 (Na⁺ salt); Calc. MS = 2885.3.
- Example 33: [Ala^{17,23,27}, 3-Pal^{19,31}]hGLP-1(7-34)-NH₂; MS = 2907.0; Calc. MS = 2901.3.
- Example 34: [D-Ala⁸, Ala^{17,23,27}, 3-Pal^{19,31}, Tie²⁹]hGLP-1(7-34)-NH₂; MS = 2906.0; Calc. MS = 2901.3.
- 20 Example 35: [D-Ala⁸, Ala^{17,23,27}, 3-Pal^{19,31}, Tie¹⁶]hGLP-1(7-34)-NH₂; MS = 2914.0; Calc. MS = 2915.4.
- Example 36: [D-Ala⁸, Ala^{17,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = 2856.8; Calc. MS = 2858.2.
- 25 Example 37: [D-Ala²², Ala^{17,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = 2871.0; Calc. MS = 2872.3.
- Example 38: [Aib⁸, Ala^{17,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = 2875.0; Calc. MS = 2872.3.
- Example 39: [D-Ala⁸, Ala^{17,22,23,27}, 3-Pal^{19,31}]hGLP-1(7-33)-NH₂; MS = 2786.0; Calc. MS = 2787.2.
- 30 Example 40: [Aib⁸, Ala^{17,22,23,27}, 3-Pal^{19,31}]hGLP-1(7-33)-NH₂; MS = 2800.0; Calc. MS = 2801.2.
- Example 41: [Ala^{17,18,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = 2842.5; Calc. MS = 2842.2.

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Example 42: [Ala^{17,23,27}, 3-Pal^{19,31}, Ile³³, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = 2871.0; Calc. MS = 2872.3.

Example 43: [Ile¹⁶, Ala^{17,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = 2870.0; Calc. MS = 2872.3.

5 Example 44: [N-Me-D-Ala⁸, Ala^{17,22,23,27}, 3-Pal^{19,31}]hGLP-1(7-33)-NH₂; MS = 2795.0; Calc. MS = 2801.2.

Example 45: [Aib⁸, Ala^{17,18,22,23,27}, 3-Pal^{19,31}]hGLP-1(7-33)-NH₂; MS = 2784.2; Calc. MS = 2785.2.

10 Example 46: [Ala^{17,18,22,23,27}, 3-Pal^{19,31}, Ile^{16,20}, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = 2871.9; Calc. MS = 2870.3.

Example 47: [D-Ala⁸, Ala^{17,18,22,23,27}, 3-Pal^{19,31}, Ile¹⁶, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = 2870.0; Calc. MS = 2870.3.

Example 48: [D-Ala^{8,22}, Ala^{17,18,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = 2856.3; Calc. MS = 2856.3.

15 Example 49: [D-Ala^{8,18}, Ala^{17,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = NA.

Example 50: [D-Ala^{8,17}, Ala^{18,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = NA.

Example 51: [D-Ala⁸, Ala^{17,18,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; MS = 2861.6; Calc. MS = 2856.3.

Example 52

20 [Aib⁸, A6c³²]hGLP-1(7-36)NH₂

The title peptide was synthesized on an Applied Biosystems (Foster City, CA) model 430A peptide synthesizer which was modified to do accelerated Boc-chemistry solid phase peptide synthesis. See Schnoize, et al., Int. J. Peptide Protein Res., 90:180 (1992). 4-Methylbenzhydrylamine (MBHA) resin (Peninsula, Belmont, CA) with the substitution of 0.93 mmol/g was used. The Boc amino acids (Bachem, CA, Torrance, CA; Nova Biochem., LaJolla, CA) were used with the following side chain protection: Boc-Ala-OH, Boc-Arg(Tos)-OH, Boc-Asp(OcHex)-OH, Boc-Tyr(2BrZ)-OH, Boc-His(DNP)-OH, Boc-Val-OH, Boc-Leu-OH, Boc-Gly-OH, Boc-Gln-OH, Boc-Ile-OH, Boc-Lys(2CIZ)-OH, Boc-Thr(Bzl)-OH, Boc-A6c-OH, Ser(Bzl)-OH, Boc-Phe-OH, Boc-Aib-OH, Boc-Glu(OcHex)-OH and Boc-Trp(Fm)-OH.

25 The synthesis was carried out on a 0.20 mmol scale. The Boc groups were removed by treatment with 100% TFA for 2 x 1 min. Boc amino acids (2.5 mmol) were pre-activated with HBTU (2.0 mmol) and DIEA (1.0 ml) in 4 ml of DMF and were coupled without prior neutralization of the peptide-resin TFA salt. Coupling times were about 5 min except for the Boc-Aib-OH and Boc-A6c-OH residues and the following residues, Boc-Trp(Fm)-OH and

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Boc-His(DNP)-OH wherein the coupling times were about 2 hours.

At the end of the assembly of the peptide chain, the resin was treated with a solution of 20% mercaptoethanol/10% DIEA in DMF for 2 x 30 min to remove the DNP group on the His side chain. The N-terminal Boc group was then removed by treatment with 100% TFA for 2 x 2 min. After neutralization of the peptide-resin with 10% DIEA in DMF (1 x 1 min), the formyl group on the side of the chain of Trp was removed by treatment with a solution of 15% ethanolamine/ 15% water/ 70% DMF for 2 x 30 min. The partially-deprotected peptide-resin was washed with DMF and DCM and dried under reduced pressure. The final cleavage was done by stirring the peptide-resin in 10 ml of HF containing 1 ml of anisole and dithiothreitol (24 mg) at 0 °C for about 75 min. HF was removed with a flow of nitrogen. The residue was washed with ether (6 x 10 ml) and extracted with 4N HOAc (6 x 10 ml).

The peptide mixture in the aqueous extract was purified on a reverse-phase preparative high pressure liquid chromatography (HPLC) using a reverse phase VYDAC® C₁₈ column (Nest Group, Southborough, MA). The column was eluted with a linear gradient (20% to 50% of solution B over 105 min) at a flow rate of 10 ml/min (Solution A = water containing 0.1% TFA; Solution B = acetonitrile containing 0.1% of TFA). Fractions were collected and checked on analytical HPLC. Those containing pure product were combined and lyophilized to dryness. 92 mg of a white solid was obtained. Purity was >99% based on analytical HPLC analysis. Electro-spray mass spectrometer analysis gave the molecular weight at 3324.2 (the calculated molecular weight is 3323.7).

The synthesis of other compounds of the present invention can be carried out in the same manner as described for the synthesis of [Aib⁸, A6c³²]hGLP-1(7-36)NH₂ in Example 52 above but using the appropriate protected amino acids depending on the desired peptide.

25 [(N^ε-HEPES-His)⁷]hGLP-1(7-36)NH₂ {HEPES is (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid)} can be synthesized as follows: After assembly of the peptide long chain on MBHA resin (0.20 mmol), the peptide-resin is treated with 100% TFA (2 x 2 min.) and washed with DMF and DCM. The resin is then neutralized with 10% DIEA in DMF for about 2 min. After washing with DMF and DCM, the resin is treated with 0.23 mmol of 2-chloro-1-ethanesulfonyl chloride and 0.7 mmol of DIEA in DMF for about 1 hour. The resin is washed with DMF and DCM and treated with 1.2 mmol of 2-hydroxyethylpiperazine for about 2 hours. The resin is washed with DMF and DCM and treated with different reagents ((1) 20% mercaptoethanol / 10% DIEA in DMF and (2) 15% ethanolamine / 15% water / 70% DMF) to remove the DNP group from the His side chain and formyl group on the Trp

side chain as described above before the final HF cleavage of the peptide from the resin.

- 5 [(N^α-HEPA-His)⁷]hGLP-1(7-36)NH₂ ([[(4-(2-hydroxyethyl)-1-piperazineacetyl)-His⁷]hGLP-1(7-36)NH₂]) can be made substantially according to the procedure described immediately above for making [(N^α-HEPES-His)⁷]hGLP-1(7-36)NH₂ except that 2-bromoacetic anhydride is used in place of 2-chloro-1-ethanesulfonyl chloride.

Examples 53-90

Examples 53-90 were made substantially according to Example 52 but using the appropriate protected amino acid.

- Example 53: [A6c^{20,32}]hGLP-1(7-36)-NH₂; MS = 3322.3; Calc. MW = 3321.7.
- 10 Example 54: [Aib⁸]hGLP-1(7-36)-NH₂; MS = 3311.7; Calc. MW = 3311.7.
- Example 55: [(Tma-His)⁷]hGLP-1(7-36)-NH₂; MS = 3395.9; Calc. MW = 3396.9.
- Example 56: [A6c⁸]hGLP-1(8-36)-NH₂; MS = 3214.5; Calc. MW = 3214.7.
- Example 57: [A6c⁸]hGLP-1(7-36)-NH₂; MS = 3351.5; Calc. MW = 3351.8.
- Example 58: [A6c^{16,20}]hGLP-1(7-36)-NH₂; MS = 3335.9; Calc. MW = 3335.8.
- 15 Example 59: [A6c^{29,32}]hGLP-1(7-36)-NH₂; MS = 3321.7; Calc. MW = 3321.7.
- Example 60: [A6c²⁰, Aib²⁴]hGLP-1(7-36)-NH₂; MS = 3323.6; Calc. MW = 3323.7.
- Example 61: [Aib²⁴, A6c^{29,32}]hGLP-1(7-36)-NH₂; MS = 3335.7; Calc. MW = 3335.8.
- Example 62: [A6c^{16,29,32}]hGLP-1(7-36)-NH₂; MS = 3347.7; Calc. MW = 3347.8.
- Example 63: [Ura⁷]hGLP-1(7-36)-NH₂; MS = 3279.5; Calc. MW = 3280.7.
- 20 Example 64: [Paa⁷]hGLP-1(7-36)-NH₂; MS = 3290.9; Calc. MW = 3291.8.
- Example 65: [Pta⁷]hGLP-1(7-36)-NH₂; MS = 3311.2; Calc. MW = 3311.8.
- Example 66: [N-Me-Ala⁸]hGLP-1(7-36)-NH₂; MS = 3311.4; Calc. MW = 3311.7.
- Example 67: [N-Me-D-Ala⁸]hGLP-1(7-36)-NH₂; MS = 3311.6; Calc. MW = 3311.7.
- Example 68: [N-Me-D-Ala⁸]hGLP-1(8-36)-NH₂; MS = 3174.0; Calc. MW = 3174.6.
- 25 Example 69: [N-Me-Gly⁸]hGLP-1(7-36)-NH₂; MS = 3297.3; Calc. MW = 3297.7.
- Example 70: [A5c⁸]hGLP-1(7-36); MS = 3337.3; Calc. MW = 3337.8.
- Example 71: [N-Me-Glu⁹]hGLP-1(7-36)-NH₂; MS = 3311.4; Calc. MW = 3311.7.
- Example 72: [A5c⁸, A6c^{20,32}]hGLP-1(7-36)-NH₂; MS = 3361.4; Calc. MW = 3361.8.
- Example 73: [Aib⁸, A6c³²]hGLP-1(7-36)-NH₂; MS = 3323.2; Calc. MW = 3323.7.
- 30 Example 74: [Aib^{8,25}]hGLP-1(7-36)-NH₂; MS = 3325.8; Calc. MW = 3325.7.
- Example 75: [Aib^{8,24}]hGLP-1(7-36)-NH₂; MS = 3325.8; Calc. MW = 3325.7.
- Example 76: [Aib^{8,30}]hGLP-1(7-36)-NH₂; MS = 3326.1; Calc. MW = 3325.7.
- Example 77: [Aib⁸, Cha²⁰]hGLP-1(7-36)-NH₂; MS = 3351.8; Calc. MW = 3351.8.
- Example 78: [Aib⁸, Cha³²]hGLP-1(7-36)-NH₂; MS = 3352.0; Calc. MW = 3351.8.

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Example 79: [Aib⁸, Glu²³]hGLP-1(7-36)-NH₂; MS = 3311.7; Calc. MW = 3312.7.

Example 80: [Aib⁸, A6c²⁰]hGLP-1(7-36)-NH₂; MS = 3323.6; Calc. MW = 3323.7.

Example 81: [Aib⁸, A6c^{20,32}]hGLP-1(7-36)-NH₂; MS = 3335.3; Calc. MW = 3335.7.

Example 82: [Aib^{8,22}]hGLP-1(7-36)-NH₂; MS = 3339.8; Calc. MW = 3339.8.

5 Example 83: [Aib⁸, β-Ala²²]hGLP-1(7-36)-NH₂; MS = 3325.6; Calc. MW = 3325.8.

Example 84: [Aib⁸, Lys²⁵]hGLP-1(7-36)-NH₂; MS = 3369.0; Calc. MW = 3368.8.

Example 85: [Aib⁸, A6c¹²]hGLP-1(7-36)-NH₂; MS = 3289.8; Calc. MW = 3289.7.

Example 86: [Aib⁸, A6c²⁹]hGLP-1(7-36)-NH₂; MS = 3323.9; Calc. MW = 3323.7.

Example 87: [Aib⁸, A6c³³]hGLP-1(7-36)-NH₂; MS = 3338.0; Calc. MW = 3337.8.

10 Example 88: [Aib^{8,14}]hGLP-1(7-36)NH₂; MS = 3309.8; Calc. MW = 3309.7.

Example 89: [Aib^{8,18}]hGLP-1(7-36)NH₂; MS = 3309.7; Calc. MW = 3309.7.

Example 90: [Aib^{8,17}]hGLP-1(7-36)NH₂; MS = 3309.4; Calc. MW = 3309.7.

Example 91

[Aib⁸, A5c³³]hGLP-1 (7-36)NH₂

15 The title compound can be made substantially according to Example 52 using the appropriate protected amino acids.

Example 92

[Aib⁸, A6c³², Lys³⁶(N^ε-tetradecanoyl)]hGLP-1(7-36)NH₂

20 The Boc amino acids to be used are the same as those in the synthesis of [Aib⁸, A6c³²]hGLP-1(7-36)NH₂ (Example 52) except that Fmoc-Lys(Boc)-OH is used here for the Lys³⁶(N^ε-tetradecanoyl) residue. The first amino acid residue is coupled to the resin manually on a shaker. 2.5 mmol of Fmoc-Lys(Boc)-OH is dissolved in 4 ml of 0.5N HBTU in DMF. To the solution is added 1 ml of DIEA. The mixture is shaken for about 2 min. To the solution is then added 0.2 mmol of MBHA resin (substitution = 0.93 mmol/g). The mixture is
25 shaken for about 1 hr. The resin is washed with DMF and treated with 100% TFA for 2x2 min to remove the Boc protecting group. The resin is washed with DMF. Myristic acid (2.5 mmol) is pre-activated with HBTU (2.0 mmol) and DIEA (1.0 ml) in 4 ml of DMF for 2 min and is coupled to the Fmoc-Lys-resin. The coupling time is about 1 hr. The resin is washed with DMF and treated with 25% piperidine in DMF for 2x20 min to remove the Fmoc
30 protecting group. The resin is washed with DMF and transferred to the reaction vessel of the peptide synthesizer. The remainder of the synthesis and purification procedures of the peptide are the same as those in the synthesis of [Aib⁸, A6c³²]hGLP-1(7-36)NH₂.

The syntheses of other compounds containing Lys(N^ε-alkanoyl) residue are carried out in an analogous manner as described for the synthesis of [Aib⁸, A6c³², Lys³⁶(N^ε-

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octanoyl)]hGLP-1(7-36)NH₂. Fmoc-Lys(Boc)-OH amino acid is used for the residue of Lys(N^ε-alkanoyl) in the peptide, while Boc-Lys(2ClZ)-OH amino acid is used for the residue of Lys. If the Lys(N^ε-alkanoyl) residue is not at the C-terminus, the peptide fragment immediately prior to the Lys(N^ε-alkanoyl) residue is assembled on the resin on the peptide synthesizer first.

Examples 93-98

Examples 93-98 can be made substantially according to the procedure described for Example 92 using the appropriate amino acids.

Example 93: [Aib⁸, A6c³², Lys³⁶(N^ε-tetradecanoyl)]hGLP-1(7-36)NH₂

Example 94: [Aib⁸, Arg^{26,34}, A6c³², Lys³⁶(N^ε-tetradecanoyl)]hGLP-1(7-36)NH₂

Example 95: [Aib⁸, Arg²⁶, A6c³², Lys³⁴(N^ε-tetradecanoyl)]hGLP-1(7-36)NH₂

Example 96: [Aib⁸, Lys²⁶(N^ε-tetradecanoyl), A6c³², Arg³⁴]hGLP-1(7-36)NH₂

Example 97: [Aib⁸, Lys³⁶(N^ε-octanoyl)]hGLP-1 (7-36)NH₂

Example 98: [Aib⁸, A6c^{20,32}, Lys³⁶(N^ε-octanoyl)]hGLP-1 (7-36)NH₂

Example 99

[Aib⁸, Arg^{26,34}, A6c³², Lys³⁶(N^ε-tetradecanoyl)]hGLP-1(7-36)-OH

The Boc amino acids to be used are the same as those used in the synthesis of [Aib⁸, A6c³², Lys³⁶(N^ε-tetradecanoyl)]hGLP-1(7-36)NH₂ (Example 92). Fmoc-Lys(Boc)-OH (2.5 mmol) is pre-activated with HBTU (2.0 mmol), HOBT (2.0 mmol) and DIEA (2.5 ml) in DMF (4 ml) for about 2 min. This amino acid is coupled to 235 mg of PAM resin (Chem-Impex, Wood Dale, IL; substitution = 0.85 mmol/g) manually on a shaker. The coupling time is about 8 hrs. The remainder of the synthesis and purification procedures for making the peptide are the same as those described in Example 52.

The syntheses of other analogs of hGLP-1(7-36)-OH and hGLP-1(7-37)-OH, which contain Lys(N^ε-alkanoyl) residue, are carried out in an analogous manner as described for the synthesis of [Aib⁸, Arg^{26,34}, A6c³², Lys³⁶(N^ε-tetradecanoyl)]hGLP-1(7-36)-OH. Fmoc-Lys(Boc)-OH amino acid is used for the residue of Lys(N^ε-alkanoyl) in the peptide, while Boc-Lys(2ClZ)-OH amino acid is used for the residue of Lys.

Examples 100-103

Examples 100-103 can be made substantially according to the procedure described for Example 99 using the appropriate amino acids.

Example 100: [Aib⁸, Arg²⁶, A6c³², Lys³⁴(N^ε-tetradecanoyl)]hGLP-1(7-36)-OH

Example 101: [Aib⁸, Lys²⁶(N^ε-tetradecanoyl), A6c³², Arg³⁴]hGLP-1(7-36)-OH

Example 102: [Aib⁸, Arg^{26,34}, A6c³², Lys³⁶(N^ε-tetradecanoyl)]hGLP-1(7-37)-OH

Example 103: [Aib⁸, Arg²⁶, A6c³², Lys³⁴(N^ε-tetradecanoyl)]hGLP-1(7-37)-OH

CLAIMS

What is claimed is:

1. A compound of formula (I),
- 5 $(R^2R^3)-A^7-A^8-A^9-A^{10}-A^{11}-A^{12}-A^{13}-A^{14}-A^{15}-A^{16}-A^{17}-A^{18}-A^{19}-A^{20}-A^{21}-A^{22}-A^{23}-A^{24}-A^{25}-A^{26}-A^{27}-A^{28}-A^{29}-A^{30}-A^{31}-A^{32}-A^{33}-A^{34}-A^{35}-A^{36}-A^{37}-R^1,$
- (I)
- wherein
- A^7 is L-His, Ura, Paa, Pta, D-His, Tyr, 3-Pal, 4-Pal, Hppa, Tma-His, Amp or deleted,
- 10 provided that when A^7 is Ura, Paa, Pta or Hppa then R^2 and R^3 are deleted;
- A^8 is Ala, D-Ala, Aib, Acc, N-Me-Ala, N-Me-D-Ala, Arg or N-Me-Gly;
- A^9 is Glu, N-Me-Glu, N-Me-Asp or Asp;
- A^{10} is Gly, Acc, Ala, D-Ala, Phe or Aib;
- A^{11} is Thr or Ser;
- 15 A^{12} is Phe, Acc, Aic, Aib, 3-Pal, 4-Pal, β -Nal, Cha, Trp or X^1 -Phe;
- A^{13} is Thr or Ser;
- A^{14} is Ser, Thr, Ala or Aib;
- A^{15} is Asp, Ala, D-Asp or Glu;
- A^{16} is Val, D-Val, Acc, Aib, Leu, Ile, Tle, Nle, Abu, Ala, D-Ala, Tba or Cha;
- 20 A^{17} is Ser, Ala, D-Ala, Aib, Acc or Thr;
- A^{18} is Ser, Ala, D-Ala, Aib, Acc or Thr;
- A^{19} is Tyr, D-Tyr, Cha, Phe, 3-Pal, 4-Pal, Acc, β -Nal, Amp or X^1 -Phe;
- A^{20} is Leu, Ala, Acc, Aib, Nle, Ile, Cha, Tle, Val, Phe or X^1 -Phe;
- A^{21} is Glu, Ala or Asp;
- 25 A^{22} is Gly, Acc, Ala, D-Ala, β -Ala or Aib;
- A^{23} is Gln, Asp, Ala, D-Ala, Aib, Acc, Asn or Glu;
- A^{24} is Ala, Aib, Val, Abu, Tle or Acc;
- A^{25} is Ala, Aib, Val, Abu, Tle, Acc, Lys, Arg, hArg, Om, $HN-CH((CH_2)_n-NR^{10}R^{11})-C(O)$ or $HN-CH((CH_2)_e-X^3)-C(O)$;
- 30 A^{26} is Lys, Ala, 3-Pal, 4-Pal, Arg, hArg, Om, Amp, $HN-CH((CH_2)_n-NR^{10}R^{11})-C(O)$ or $HN-CH((CH_2)_e-X^3)-C(O)$;
- A^{27} is Glu, Ala, D-Ala or Asp;
- A^{28} is Phe, Ala, Pal, β -Nal, X^1 -Phe, Aic, Acc, Aib, Cha or Trp;
- A^{29} is Ile, Acc, Aib, Leu, Nle, Cha, Tle, Val, Abu, Ala, Tba or Phe;

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A³⁰ is Ala, Aib, Acc or deleted;

A³¹ is Trp, Ala, β-Nal, 3-Pal, 4-Pal, Phe, Acc, Aib, Cha, Amp or deleted;

A³² is Leu, Ala, Acc, Aib, Nle, Ile, Cha, Tie, Phe, X¹-Phe, Ala or deleted;

A³³ is Val, Acc, Aib, Leu, Ile, Tie, Nle, Cha, Ala, Phe, Abu, X¹-Phe, Tba, Gaba or deleted;

5 A³⁴ is Lys, Arg, hArg, Orn, Amp, Gaba, HN-CH((CH₂)_n-NR¹⁰R¹¹)-C(O), HN-CH((CH₂)_e-X³)-C(O) or deleted;

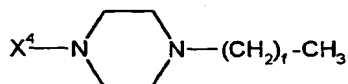
A³⁵ is Gly or deleted;

A³⁶ is L- or D-Arg, D- or L-Lys, D- or L-hArg, D- or L-Orn, Amp, HN-CH((CH₂)_n-NR¹⁰R¹¹)-C(O), HN-CH((CH₂)_e-X³)-C(O) or deleted;

10 A³⁷ is Gly or deleted;

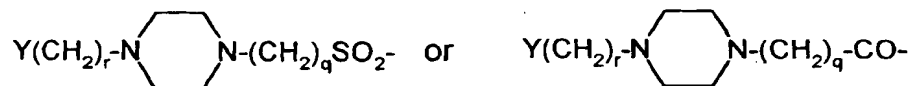
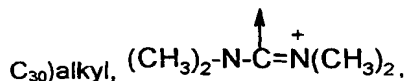
X¹ for each occurrence is independently selected from the group consisting of (C₁-C₆)alkyl, OH and halo;

R¹ is OH, NH₂, (C₁-C₁₂)alkoxy, or NH-X²-CH₂-Z, wherein X² is a (C₁-C₁₂)hydrocarbon moiety, and Z is H, OH, CO₂H or CONH₂;



15 X³ is or -C(O)-NHR¹², wherein X⁴ for each occurrence is independently -C(O)-, -NH-C(O)- or -CH₂-, and f for each occurrence is independently an integer from 1 to 29;

each of R² and R³ is independently selected from the group consisting of H, (C₁-C₃₀)alkyl, (C₂-C₃₀)alkenyl, phenyl(C₁-C₃₀)alkyl, naphthyl(C₁-C₃₀)alkyl, hydroxy(C₁-C₃₀)alkyl, hydroxy(C₂-C₃₀)alkenyl, hydroxyphenyl(C₁-C₃₀)alkyl, and hydroxynaphthyl(C₁-C₃₀)alkyl; or one of R² and R³ is C(O)X⁵ in which X⁵ is (C₁-C₃₀)alkyl, (C₂-C₃₀)alkenyl, phenyl(C₁-C₃₀)alkyl, naphthyl(C₁-C₃₀)alkyl, hydroxy(C₁-C₃₀)alkyl, hydroxy(C₂-C₃₀)alkenyl, hydroxyphenyl(C₁-C₃₀)alkyl, hydroxynaphthyl(C₁-



25

(a)

(b)

where Y is H or OH, r is 0 to 4 and q is 0 to 4;

e for each occurrence is independently an integer from 1 to 4;

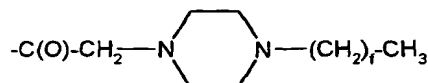
n for each occurrence is independently an integer from 1-5; and

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R¹⁰ and R¹¹ for each occurrence is each independently H, (C₁-C₃₀)alkyl, (C₁-C₃₀)acyl, (C₁-C₃₀)alkylsulfonyl, -C((NH)(NH₂)) or



, provided that when R¹⁰ is (C₁-C₃₀)acyl, (C₁-



C₃₀)alkylsulfonyl, -C((NH)(NH₂)) or

, R¹¹ is H or

(C₁-C₃₀)alkyl; and

R¹² is (C₁-C₃₀)alkyl;

with the proviso that:

- (i) at least one amino acid of a compound of formula (I) is not the same as the native sequence of hGLP-1(7-36, or -37)NH₂ or hGLP-1(7-36, or -37)OH;
- 10 (ii) a compound of formula (I) is not an analogue of hGLP-1(7-36, or -37)NH₂ or hGLP-1(7-36, or -37)OH wherein a single position has been substituted by Ala;
- (iii) a compound of formula (I) is not [Lys²⁶(N^ε-alkanoyl)]hGLP-1(7-36, or -37)-E, [Lys³⁴(N^ε-alkanoyl)]hGLP-1(7-36, or -37)-E, [Lys^{26,34}-bis(N^ε-alkanoyl)]hGLP-1(7-36, or -37)-E, [Arg²⁶, Lys³⁴(N^ε-alkanoyl)]hGLP-1(8-36, or -37)-E, or [Arg^{26,34}, Lys³⁶(N^ε-alkanoyl)]hGLP-1(7-36, or
- 15 -37)-E, wherein E is -OH or -NH₂;
- (iv) a compound of formula (I) is not Z-hGLP-1(7-36, or -37)-OH, Z-hGLP-1(7-36, or -37)-NH₂, where Z is selected from the group consisting of
- (e) [Arg²⁶], [Arg³⁴], [Arg^{26,34}], [Lys³⁶], [Arg²⁶, Lys³⁶], [Arg³⁴, Lys³⁶], [D-Lys³⁶], [Arg³⁶], [D-Arg³⁶], [Arg^{26,34}, Lys³⁶] or [Arg^{26,36}, Lys³⁴];
- 20 (f) [Asp²¹];
- (g) at least one of [Aib⁸], [D-Ala⁸] and [Asp⁹]; and
- (h) [Tyr⁷], [N-acyl-His⁷], [N-alkyl-His⁷], [N-acyl-D-His⁷] or [N-alkyl-D-His⁷];
- (v) a compound of formula (I) is not a combination of any two of the substitutions listed in groups (a) to (d); and
- 25 (vi) a compound of formula (I) is not [N-Me-Ala⁸]hGLP-1(8-36 or -37), [Glu¹⁵]hGLP-1(7-36 or -37), [Asp²¹]hGLP-1(7-36 or -37) or [Phe³¹]hGLP-1(7-36 or -37).

2. A compound according to claim 1 or a pharmaceutically acceptable salt thereof wherein A¹¹ is Thr; A¹³ is Thr; A¹⁴ is Ser, Aib or Ala; A¹⁷ is Ser, Ala, Aib or D-Ala; A¹⁸ is Ser, Ala, Aib or D-Ala; A²¹ is Glu or Ala; A²³ is Gln, Glu, or Ala; and A²⁷ is Glu or Ala.

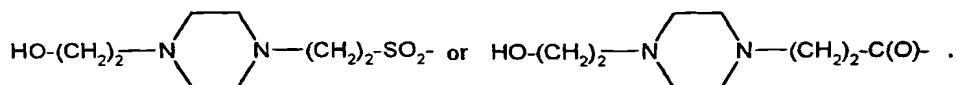
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3. A compound according to claim 2 or a pharmaceutically acceptable salt thereof wherein A⁹ is Glu, N-Me-Glu or N-Me-Asp; A¹² is Phe, Acc or Aic; A¹⁶ is Val, D-Val, Acc, Aib, Ala, Tle or D-Ala; A¹⁹ is Tyr, 3-Pal, 4-Pal or D-Tyr; A²⁰ is Leu, Acc, Cha, Ala or Tle; A²⁴ is Ala, Aib or Acc; A²⁵ is Ala, Aib, Acc, Lys, Arg, hArg, Orn, HN-CH((CH₂)_n-NH-R¹⁰)-C(O); A²⁸ is Phe or Ala; A²⁹ is Ile, Acc or Tle; A³⁰ is Ala, Aib or deleted; A³¹ is Trp, Ala, 3-Pal, 4-Pal or deleted; A³² is Leu, Acc, Cha, Ala or deleted; A³³ is Val, Acc, Ala, Gaba, Tle or deleted.

4. A compound according to claim 3 or a pharmaceutically acceptable salt thereof wherein A⁸ is Ala, D-Ala, Aib, A6c, A5c, N-Me-Ala, N-Me-D-Ala or N-Me-Gly; A¹⁰ is Gly, Ala, D-Ala or Phe; A¹² is Phe, A6c or A5c; A¹⁶ is Val, Ala, Tle, A6c, A5c or D-Val; A²⁰ is Leu, A6c, A5c, Cha, Ala or Tle; A²² is Gly, Aib, β-Ala, L-Ala or D-Ala; A²⁴ is Ala or Aib; A²⁹ is Ile, A6c, A5c or Tle; A³² is Leu, A6c, A5c, Cha, Ala or deleted; A³³ is Val, A6c, A5c, Ala, Gaba, Tle or deleted.

5. A compound according to claim 4 or a pharmaceutically acceptable salt thereof wherein R¹ is OH or NH₂.

6. A compound according to claim 5 or a pharmaceutically acceptable salt thereof wherein R² is H and R³ is (C₁-C₃₀)alkyl, (C₂-C₃₀)alkenyl, (C₁-C₃₀)acyl,



7. A compound according to claim 1 wherein said compound is

[D-Ala⁸, Ala^{17,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]-GLP-1(7-34)NH₂;

[D-Ala^{8,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂;

[Ala^{18,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂;

[Ala^{16,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂;

[Ala^{14,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂;

[Ala^{22,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂;

[Hppa⁷]hGLP-1(7-36)-NH₂;

[Ala^{15,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂;

[Ala^{17,23,27}, 3-Pal^{19,31}]hGLP-1(7-35)-NH₂;

[Ala^{22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂;

[Ala^{15,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂;

[Ala^{17,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂;

[Ala^{18,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂;

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- [Ala^{21,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂;
 [Ala^{22,23,26,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂;
 [Ala^{22,23,27,32}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂;
 [Ala^{22,23,26,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂;
 5 [Ala^{22,23,27,31}, 3-Pal¹⁹, Gaba³³]hGLP-1(7-33)-NH₂;
 [Ala^{22,23,27,28}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂;
 [Ala^{22,23,27,29}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂;
 [Ala^{23,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂;
 [Ala^{20,22,23,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂;
 10 [Ala^{22,23,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂;
 [Ala^{17,22,23,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂;
 [D-Ala¹⁰, Ala^{22,23,27}, 3-Pal^{19,31}, Gaba³³]hGLP-1(7-33)-NH₂;
 [D-Ala⁸, Ala^{17,23,27}, 3-Pal^{19,31}]hGLP-1(7-34)-NH₂;
 [Ala^{17,23,27}, 3-Pal^{19,26,31}]hGLP-1(7-34)-NH₂;
 15 [D-Ala⁸, Ala¹⁷, 3-Pal^{19,31}]hGLP-1(7-34)-NH₂;
 [Ala^{17,23,27}, 3-Pal^{19,31}]hGLP-1(7-34)-NH₂;
 [D-Ala⁸, Ala^{17,23,27}, 3-Pal^{19,31}, Tie²⁹]hGLP-1(7-34)-NH₂;
 [D-Ala⁸, Ala^{17,23,27}, 3-Pal^{19,31}, Tie¹⁶]hGLP-1(7-34)-NH₂;
 [D-Ala⁸, Ala^{17,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂;
 20 [D-Ala²², Ala^{17,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂;
 [Aib⁸, Ala^{17,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂;
 [D-Ala⁸, Ala^{17,22,23,27}, 3-Pal^{19,31}]hGLP-1(7-33)-NH₂;
 [Aib⁸, Ala^{17,22,23,27}, 3-Pal^{19,31}]hGLP-1(7-33)-NH₂;
 [Ala^{17,18,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂;
 25 [Ala^{17,23,27}, 3-Pal^{19,31}, Tie³³, Gaba³⁴]hGLP-1(7-34)-NH₂;
 [Tie¹⁶, Ala^{17,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂;
 [N-Me-D-Ala⁸, Ala^{17,22,23,27}, 3-Pal^{19,31}]hGLP-1(7-33)-NH₂;
 [Aib⁸, Ala^{17,18,22,23,27}, 3-Pal^{19,31}]hGLP-1(7-33)-NH₂;
 [Ala^{17,18,22,23,27}, 3-Pal^{19,31}, Tie^{16,20}, Gaba³⁴]hGLP-1(7-34)-NH₂;
 30 [D-Ala⁸, Ala^{17,18,22,23,27}, 3-Pal^{19,31}, Tie¹⁶, Gaba³⁴]hGLP-1(7-34)-NH₂;
 [D-Ala^{8,22}, Ala^{17,18,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂;
 [D-Ala^{8,18}, Ala^{17,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂;
 [D-Ala^{8,17}, Ala^{18,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; or

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[D-Ala⁸, Ala^{17,18,22,23,27}, 3-Pal^{19,31}, Gaba³⁴]hGLP-1(7-34)-NH₂; or a pharmaceutically acceptable salt thereof.

8. A compound according to claim 1 wherein said compound is

- [Aib⁸, A6c³²]hGLP-1(7-36)-NH₂;
5 [A6c^{20,32}]hGLP-1(7-36)-NH₂;
[Aib⁸]hGLP-1(7-36)-NH₂;
[(Tma-His)⁷]hGLP-1(7-36)-NH₂;
[A6c⁸]hGLP-1(8-36)-NH₂;
[A6c⁸]hGLP-1(7-36)-NH₂;
10 [A6c^{16,20}]hGLP-1(7-36)-NH₂;
[A6c^{29,32}]hGLP-1(7-36)-NH₂;
[A6c²⁰, Aib²⁴]hGLP-1(7-36)-NH₂;
[Aib²⁴, A6c^{29,32}]hGLP-1(7-36)-NH₂;
[A6c^{16,29,32}]hGLP-1(7-36)-NH₂;
15 [Ura⁷]hGLP-1(7-36)-NH₂;
[Paa⁷]hGLP-1(7-36)-NH₂;
[Pta⁷]hGLP-1(7-36)-NH₂;
[N-Me-Ala⁸]hGLP-1(7-36)-NH₂;
[N-Me-D-Ala⁸]hGLP-1(7-36)-NH₂;
20 [N-Me-D-Ala⁸]hGLP-1(8-36)-NH₂;
[N-Me-Gly⁸]hGLP-1(7-36)-NH₂;
[A5c⁸]hGLP-1(7-36) ;
[N-Me-Glu⁸]hGLP-1(7-36)-NH₂;
[A5c⁸, A6c^{20,32}]hGLP-1(7-36)-NH₂;
25 [Aib⁸, A6c³²]hGLP-1(7-36)-NH₂;
[Aib^{8,25}]hGLP-1(7-36)-NH₂;
[Aib^{8,24}]hGLP-1(7-36)-NH₂;
[Aib^{8,30}]hGLP-1(7-36)-NH₂;
[Aib⁸, Cha²⁰]hGLP-1(7-36)-NH₂;
30 [Aib⁸, Cha³²]hGLP-1(7-36)-NH₂;
[Aib⁸, Glu²³]hGLP-1(7-36)-NH₂;
[Aib⁸, A6c²⁰]hGLP-1(7-36)-NH₂;
[Aib⁸, A6c^{20,32}]hGLP-1(7-36)-NH₂;
[Aib^{8,22}]hGLP-1(7-36)-NH₂;

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[Aib⁸,β-Ala²²]hGLP-1(7-36)-NH₂;

[Aib⁸, Lys²⁵]hGLP-1(7-36)-NH₂;

[Aib⁸, A6c¹²]hGLP-1(7-36)-NH₂;

[Aib⁸, A6c²⁹]hGLP-1(7-36)-NH₂;

5 [Aib⁸, A6c³³]hGLP-1(7-36)-NH₂;

[Aib^{8,14}]hGLP-1(7-36)NH₂;

[Aib^{8,16}]hGLP-1(7-36)NH₂; or

[Aib^{8,17}]hGLP-1(7-36)NH₂; or a pharmaceutically acceptable salt thereof.

9. A pharmaceutical composition comprising an effective amount of a
10 compound according to claim 1 or a pharmaceutically acceptable salt thereof and a
pharmaceutically acceptable carrier or diluent.

10. A method of eliciting an agonist effect from a GLP-1 receptor in a subject in
need thereof which comprises administering to said subject an effective amount of a
compound according to claim 1 or a pharmaceutically acceptable salt thereof.

15 11. A method of treating a disease selected from the group consisting of Type I
diabetes, Type II diabetes, obesity, glucagonomas, secretory disorders of the airway,
metabolic disorder, arthritis, osteoporosis, central nervous system disease, restenosis and
neurodegenerative disease, in a subject in need thereof which comprises administering to
20 said subject an effective amount of a compound according to claim 1 or a pharmaceutically
acceptable salt thereof.

12. A method according to claim 11 wherein said disease is Type I diabetes or
Type II diabetes.

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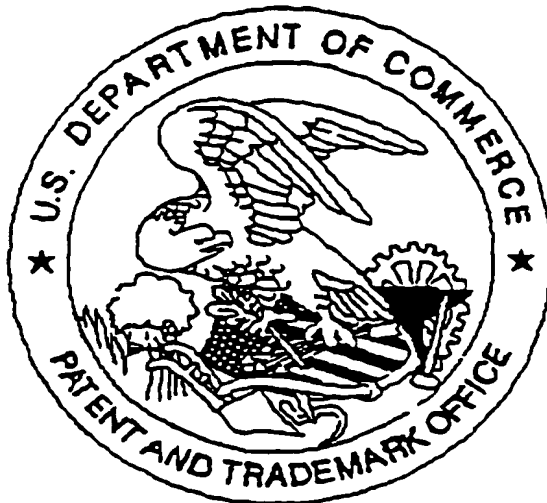
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ABSTRACT

The present invention is directed to peptide analogues of glucagon-like peptide-1, the pharmaceutically-acceptable salts thereof, to methods of using such analogues to treat
5 mammals and to pharmaceutical compositions useful therefor comprising said analogues.

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